SECTION 7

LABORATORY PROCEDURES I — SAMPLE HANDLING

This section provides guidance on laboratory procedures for sample receipt, chain-of-custody, processing, distribution, analysis, and archiving. Planning, documentation, and quality assurance and quality control of all laboratory activities are emphasized to ensure that (1) sample integrity is preserved during all phases of sample handling and analysis, (2) chemical analyses are performed cost-effectively and meet program data quality objectives, and (3) data produced by different States and Regions are comparable.

Laboratory procedures should be documented in a Work/QA Project Plan (U.S. EPA, 1980b) as described in Appendix F. Routine sample processing and analysis procedures should be prepared as standard operating procedures (SOPs) (U.S. EPA, 1984b).

7.1 SAMPLE RECEIPT AND CHAIN-OF-CUSTODY

Fish, shellfish, and turtle samples may be shipped or hand-carried from the field according to one or more of the following pathways:

- From the field to a State laboratory for sample processing and analysis
- From the field to a State laboratory for sample processing and shipment of composite sample aliquots to a contract laboratory for analysis
- From the field to a contract laboratory for sample processing and analysis.

Sample processing and distribution for analysis ideally should be performed by one processing laboratory. Transportation of samples from the field should be coordinated by the sampling team supervisor and the laboratory supervisor responsible for sample processing and distribution (see Section 6.3.4). An accurate written custody record must be maintained so that possession and treatment of each sample can be traced from the time of collection through analysis and final disposition.

Fish, shellfish, and turtle samples should be brought or shipped to the sample processing laboratory in sealed containers accompanied by a copy of the sample request form (Figure 6-1), a chain-of-custody form (Figure 6-8), and the field records (Figures 6-2 through 6-5). Each time custody of a sample or set of samples is transferred, the Personnel Custody Record of the COC form must be

completed and signed by both parties. Corrections to the COC form should be made in indelible ink by drawing a single line through the original entry, entering the correct information and the reason for the change, and initialing and dating the correction. The original entry should never be obscured.

When custody is transferred from the field to the sample processing laboratory, the following procedure should be used:

- Note the shipping time. If samples have been shipped on wet or blue ice, check that the shipping time has not exceeded 24 hours.

- Check that each shipping container has arrived undamaged and that the seal is intact.

- Open each shipping container and remove the copy of the sample request form, the COC form, and the field records.

- Note the general condition of the shipping container (samples iced properly with no leaks, etc.) and the accompanying documentation (dry, legible, etc.).

- Locate individuals in each composite sample listed on the COC form and note the condition of their packaging. Individual specimens should be properly wrapped and labeled. Note any problems (container punctured, illegible labels, etc.) on the COC form.

- If individuals in a composite are packaged together, check the contents of each composite sample container against the field record for that sample to ensure that the individual specimens are properly wrapped and labeled. Note any discrepancies or missing information on the COC form.

- Initial the COC form and record the date and time of sample receipt.

- Enter the following information for each composite sample into a permanent laboratory record book and, if applicable, a computer database:

  — Sample identification number (specify conventions for the composite sample number and the specimen number) **Note:** EPA recommends processing and analysis of turtles as individual samples.

  — Receipt date (specify convention, e.g., day/month/year)

  — Sampling date (specify convention, e.g., day/month/year)

  — Sampling site (name and/or identification number)

  — Fish, turtle, and shellfish species (scientific name or code number)

— Total length of each fish, carapace length of each turtle, or size of each shellfish (mm)

- If samples have been shipped on wet or blue ice, distribute them immediately to the technician responsible for resection (see Section 7.2). See Section 7.2.3 for the procedure for processing turtle samples as individual samples. If samples have been shipped on dry ice, they may be distributed immediately to the technician for processing or stored in a freezer at ≤-20 °C for later processing. Once processed, fillets or edible portions of fish, turtles or shellfish, or tissue homogenates, should be stored according to the procedures described in Section 7.2 and in Table 7-1. **Note**: Holding times in Table 7-1 are maximum times recommended for holding samples from the time they are received at the laboratory until they are analyzed. These holding times are based on guidance that is sometimes administrative rather than technical in nature; there are no promulgated holding time criteria for tissues (U.S. EPA, 1995k). If States choose to use longer holding times, they must demonstrate and document the stability of the target analyte residues over the extended holding times.

7.2 SAMPLE PROCESSING

This section includes recommended procedures for preparing composite homogenate samples of fish fillets and edible portions of shellfish and individual samples of edible portions of freshwater turtles as required in screening and intensive studies. Recommended procedures for preparing whole fish composite homogenates are included in Appendix G for use by States in assessing the potential risk to local subpopulations known to consume whole fish or shellfish.

7.2.1 General Considerations

All laboratory personnel performing sample processing procedures (see Sections 7.2.2, 7.2.3, and 7.2.4) should be trained or supervised by an experienced fisheries biologist. Care must be taken during sample processing to avoid contaminating samples. Schmitt and Finger (1987) have demonstrated that contamination of fish flesh samples is likely unless the most exacting clean dissection procedures are used. Potential sources of contamination include dust, instruments, utensils, work surfaces, and containers that may contact the samples. All sample processing (i.e., filleting, removal of other edible tissue, homogenizing, compositing) should be done in an appropriate laboratory facility under cleanroom conditions (Stober, 1991). Cleanrooms or work areas should be free of metals and organic contaminants. Ideally, these areas should be under positive pressure with filtered air (HEPA filter class 100) (California Department of Fish and Game, 1990). Periodic wipe tests should be conducted in clean areas to verify the absence of significant levels of metal and organic contaminants. All instruments, work surfaces, and containers used to process samples must be of materials that can be cleaned easily and that are not themselves potential sources of contamination. More detailed guidance on establishing trace metal cleanrooms is provided in U.S. EPA (1995b).

Table 7-1. Recommendations for Container Materials, Preservation, and Holding Times for Fish, Shellfish, and Turtle Tissues from Receipt at Sample Processing Laboratory to Analysis

			Storage	
Analyte	**Matrix**	**Sample container**	**Preservation**	**Holding time[a]**
Mercury	Tissue (fillets and edible portions, homogenates)	Plastic, borosilicate glass, quartz, PTFE	Freeze at ≤-20 °C	28 days[b]
Other metals	Tissue (fillets and edible portions, homogenates)	Plastic, borosilicate glass, quartz, PTFE	Freeze at ≤-20 °C	6 months[c]
Organics	Tissue (fillets and edible portions, homogenates)	Borosilicate glass, PTFE, quartz, aluminum foil	Freeze at ≤-20 °C	1 year[d]
Metals and organics	Tissue (fillets and edible portions, homogenates)	Borosilicate glass, quartz, PTFE	Freeze at ≤-20 °C	28 days (for mercury); 6 months (for other metals); and 1 year (for organics)
Lipids	Tissue (fillets and edible portions, homogenates)	Plastic, borosilicate glass, quartz, PTFE	Freeze at ≤-20 °C	1 year

PTFE = Polytetrafluoroethylene (Teflon).

[a] Maximum holding times recommended by EPA (1995k).

[b] This maximum holding time is also recommended by the Puget Sound Estuary Program (1990e). The California Department of Fish and Game (1990) and the USGS National Water Quality Assessment Program (Crawford and Luoma, 1993) recommend a maximum holding time of 6 months for all metals, including mercury.

[c] This maximum holding time is also recommended by the California Department of Fish and Game (1990), the 301(h) monitoring program (U.S. EPA, 1986b), and the USGS National Water Quality Assessment Program (Crawford and Luoma, 1993). The Puget Sound Estuary Program (1990e) recommends a maximum holding time of 2 years.

[d] This maximum holding time is also recommended by the Puget Sound Estuary Program (1990e). The California Department of Fish and Game (1990) and the USGS National Water Quality Assessment Program (Crawford and Luoma, 1993) recommend a more conservative maximum holding time of 6 months. The EPA (1995c) recommends a maximum holding time of 1 year at ≤-10 °C for dioxins/furans.

To avoid cross-contamination, all equipment used in sample processing (i.e., resecting, homogenizing, and compositing) should be cleaned thoroughly before each composite sample is prepared. Verification of the efficacy of cleaning procedures should be documented through the analysis of processing blanks or rinsates (see Section 8.3.3.6).

Because sources of organic and metal contaminants differ, it is recommended that duplicate samples be collected, if time and funding permit, when analyses of both organics and metals are required (e.g., for screening studies). One sample can then be processed and analyzed for organics and the other can be processed independently and analyzed for metals (Batelle, 1989; California Department of Fish and Game, 1990; Puget Sound Estuary Program, 1990c, 1990d). If fish are of adequate size, separate composites of individual fillets may be prepared and analyzed independently for metals and organics. If only one composite sample is prepared for the analyses of metals and organics, the processing equipment must be chosen and cleaned carefully to avoid contamination by both organics and metals.

Suggested sample processing equipment and cleaning procedures by analysis type are discussed in Sections 7.2.1.1 through 7.2.1.3. Other procedures may be used if it can be demonstrated, through the analysis of appropriate blanks, that no contamination is introduced (see Section 8.3.3.6).

7.2.1.1 Samples for Organics Analysis—

Equipment used in processing samples for organics analysis should be of stainless steel, anodized aluminum, borosilicate glass, polytetrafluoroethylene (PTFE), ceramic, or quartz. Polypropylene and polyethylene (plastic) surfaces, implements, gloves, and containers are a potential source of contamination by organics and should not be used. If a laboratory chooses to use these materials, there should be clear documentation that they are not a source of contamination. Filleting should be done on glass or PTFE cutting boards that are cleaned properly between fish or on cutting boards covered with heavy duty aluminum foil that is changed after each filleting. Tissue should be removed with clean, high-quality, corrosion-resistant stainless steel or quartz instruments or with knives with titanium blades and PTFE handles (Lowenstein and Young, 1986). Fillets or tissue homogenates may be stored in borosilicate glass, quartz, or PTFE containers with PTFE-lined lids or in heavy duty aluminum foil (see Table 7-1).

Prior to preparing each composite sample, utensils and containers should be washed with detergent solution, rinsed with tap water, soaked in pesticide-grade isopropanol or acetone, and rinsed with organic-free, distilled, deionized water. Work surfaces should be cleaned with pesticide-grade isopropanol or acetone, washed with distilled water, and allowed to dry completely. Knives, fish scalers, measurement boards, etc., should be cleaned with pesticide-grade isopropanol

or acetone followed by a rinse with contaminant-free distilled water between each fish sample (Stober, 1991).

7.2.1.2 Samples for Metals Analysis—

Equipment used in processing samples for metals analyses should be of quartz, PTFE, ceramic, polypropylene, or polyethylene. The predominant metal contaminants from stainless steel are chromium and nickel. If these metals are not of concern, the use of high-quality, corrosion-resistant stainless steel for sample processing equipment is acceptable. Quartz utensils are ideal but expensive. For bench liners and bottles, borosilicate glass is preferred over plastic (Stober, 1991). Knives with titanium blades and PTFE handles are recommended for performing tissue resections (Lowenstein and Young, 1986). Borosilicate glass bench liners are recommended. Filleting may be done on glass or PTFE cutting boards that are cleaned properly between fish or on cutting boards covered with heavy duty aluminum foil that is changed after each fish. Fillets or tissue homogenates may be stored in plastic, borosilicate glass, quartz, or PTFE containers (see Table 7-1).

Prior to preparing each composite sample, utensils and containers should be cleaned thoroughly with a detergent solution, rinsed with tap water, soaked in acid, and then rinsed with metal-free water. Quartz, PTFE, glass, or plastic containers should be soaked in 50% $HN0_3$, for 12 to 24 hours at room temperature. **Note:** Chromic acid should not be used for cleaning any materials. Acids used should be at least reagent grade. Stainless steel parts may be cleaned as stated for glass or plastic, omitting the acid soaking step (Stober, 1991).

7.2.1.3 Samples for Both Organics and Metals Analyses—

As noted above, several established monitoring programs, including the Puget Sound Estuary Program (1990c, 1990d), the NOAA Mussel Watch Program (Battelle, 1989), and the California Mussel Watch Program (California Department of Fish and Game, 1990), recommend different procedures for processing samples for organics and metals analyses. However, this may not be feasible if fish are too small to allow for preparing separate composites from individual fillets or if resources are limited. If a single composite sample is prepared for the analyses of both organics and metals, precautions must be taken to use materials and cleaning procedures that are noncontaminating for both organics and metals.

Quartz, ceramic, borosilicate glass, and PTFE are recommended materials for sample processing equipment. If chromium and nickel are not of concern, high-quality, corrosion-resistant stainless steel utensils may be used. Knives with titanium blades and PTFE handles are recommended for performing tissue resections (Lowenstein and Young, 1986). Borosilicate glass bench liners are recommended. Filleting should be done on glass or PTFE cutting boards that are cleaned properly between fish or on cutting boards covered with heavy duty

aluminum foil that is changed after each filleting. Fillets or tissue homogenates should be stored in clean borosilicate glass, quartz, or PTFE containers with PTFE-lined lids.

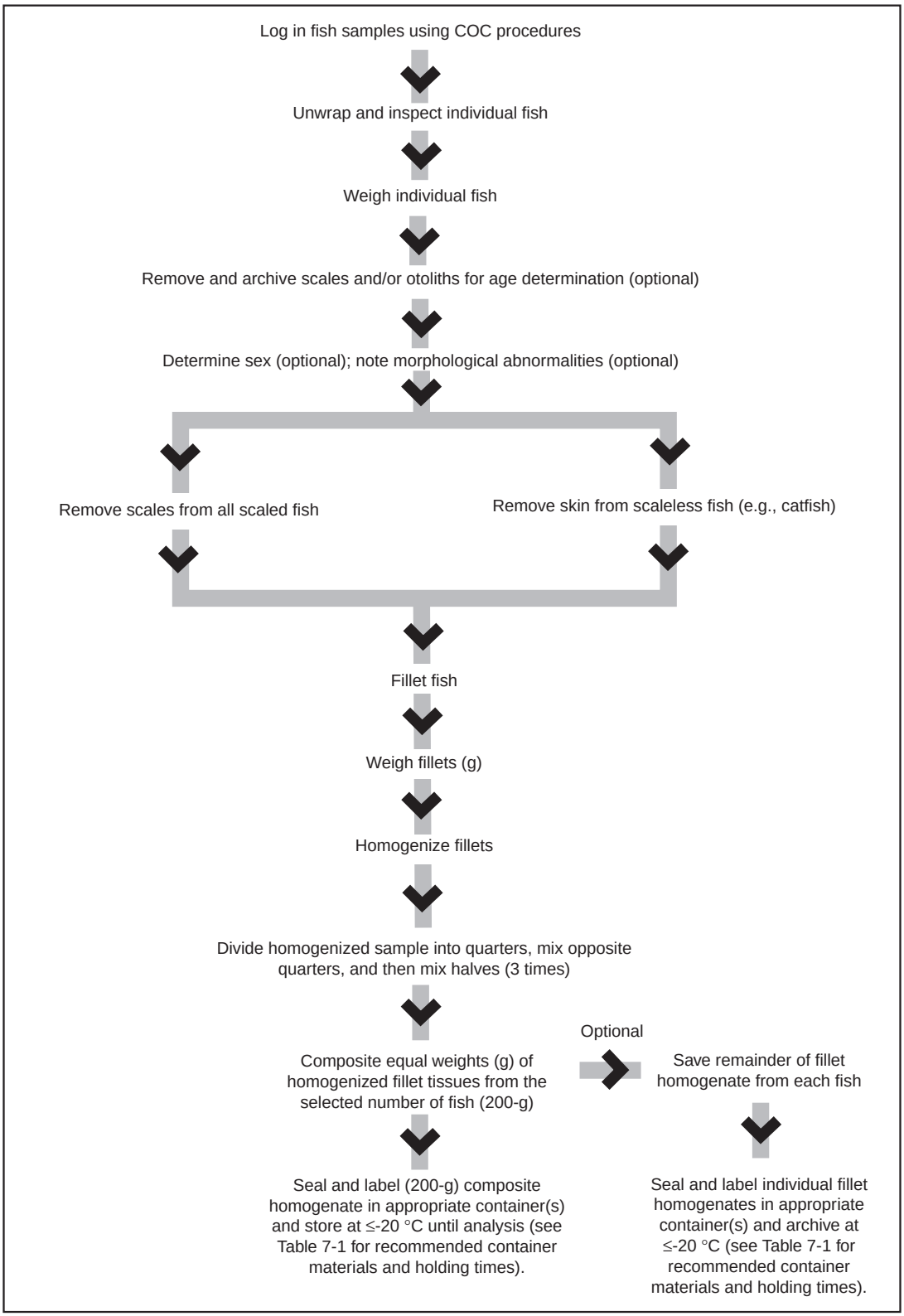
Prior to preparing each composite sample, utensils and containers should be cleaned thoroughly with a detergent solution, rinsed with tap water, soaked in 50% HNO_3, for 12 to 24 hours at room temperature, and then rinsed with organics- and metal-free water. **Note:** Chromic acid should not be used for cleaning any materials. Acids used should be at least reagent grade. Stainless steel parts may be cleaned using this recommended procedure with the acid soaking step method omitted (Stober, 1991).

Aliquots of composite homogenates taken for metals analysis (see Section 7.3.1) may be stored in plastic containers that have been cleaned according to the procedure outlined above, with the exception that aqua regia must not be used for the acid soaking step.

7.2.2 Processing Fish Samples

Processing in the laboratory to prepare fish fillet composite homogenate samples for analysis (diagrammed in Figure 7-1) involves

- Inspecting individual fish
- Weighing individual fish
- Removing scales and/or otoliths for age determination (optional)
- Determining the sex of each fish (optional)
- Examining each fish for morphological abnormalities (optional)
- Scaling all fish with scales (leaving belly flap on); removing skin of scaleless fish (e.g., catfish)
- Filleting (resection)
- Weighing fillets
- Homogenizing fillets
- Preparing a composite homogenate
- Preparing aliquots of the composite homogenate for analysis
- Distributing frozen aliquots to one or more analytical laboratories.



COC = Chain of custody.

Figure 7-1. Preparation of fish fillet composite homogenate samples.

Whole fish should be shipped or brought to the sample processing laboratory from the field on wet or blue ice within 24 hours of sample collection. Fillets should be resected within 48 hours of sample collection. Ideally, fish should not be frozen prior to resection because freezing may cause internal organs to rupture and contaminate edible tissue (Stober, 1991; U.S. EPA, 1986b). However, if resection cannot be performed within 48 hours, the whole fish should be frozen at the sampling site and shipped to the sample processing laboratory on dry ice. Fish samples that arrive frozen (i.e., on dry ice) at the sample processing laboratory should be placed in a ≤−20 °C freezer for storage until filleting can be performed. The fish should then be partially thawed prior to resection. **Note:** If the fillet tissue is contaminated by materials released from the rupture of the internal organs during freezing, the State may eliminate the fillet tissue as a sample or, alternatively, the fillet tissues should be rinsed in contaminant-free, distilled deionized water and blotted dry. Regardless of the procedure selected, a notation should be made in the sample processing record.

Sample processing procedures are discussed in the following sections. Data from each procedure should be recorded directly in a bound laboratory notebook or on forms that can be secured in the laboratory notebook. An example sample processing record for fish fillet composites is shown in Figure 7-2.

7.2.2.1 Sample Inspection—

Individual fish received for filleting should be unwrapped and inspected carefully to ensure that they have not been compromised in any way (i.e., not properly preserved during shipment). Any specimen deemed unsuitable for further processing and analysis should be discarded and identified on the sample processing record.

7.2.2.2 Sample Weighing—

A wet weight should be determined for each fish. All samples should be weighed on balances that are properly calibrated and of adequate accuracy and precision to meet program data quality objectives. Balance calibration should be checked at the beginning and end of each weighing session and after every 20 weighings in a weighing session.

Fish shipped on wet or blue ice should be weighed directly on a foil-lined balance tray. To prevent cross contamination between individual fish, the foil lining should be replaced after each weighing. Frozen fish (i.e., those shipped on dry ice) should be weighed in clean, tared, noncontaminating containers if they will thaw before the weighing can be completed. **Note:** Liquid from the thawed whole fish sample will come not only from the fillet tissue but from the gut and body cavity, which are not part of the final fillet sample. Consequently, inclusion of this liquid with the sample may result in an overestimate of target analyte and lipid concentrations in the fillet homogenate. Nevertheless, it is recommended, as a conservative approach, that all liquid from the thawed whole fish sample be kept in the container as part of the sample.

Figure 7-2. Example of a sample processing record for fish contaminant monitoring program—fish fillet composites.

All weights should be recorded to the nearest gram on the sample processing record and/or in the laboratory notebook.

7.2.2.3 Age Determination (Optional)—

Age provides a good indication of the duration of exposure to pollutants (Versar, 1982). A few scales or otoliths (Jearld, 1983) should be removed from each fish and delivered to a fisheries biologist for age determination. For most warm water inland gamefish, 5 to 10 scales should be removed from below the lateral line and behind the pectoral fin. On soft-rayed fish such as trout and salmon, the scales should be taken just above the lateral line (WDNR, 1988). For catfish and other scaleless fish, the pectoral fin spines should be clipped and saved (Versar, 1982). The scales, spines, or otoliths may be stored by sealing them in small envelopes (such as coin envelopes) or plastic bags labeled with, and cross-referenced by, the identification number assigned to the tissue specimen (Versar, 1982). Removal of scales, spines, or otoliths from each fish should be noted (by a check mark) on the sample processing record.

7.2.2.4 Sex Determination (Optional)—

Fish sex should be determined before filleting. To determine the sex of a fish, an incision should be made on the ventral surface of the body from a point immediately anterior to the anus toward the head to a point immediately posterior to the pelvic fins. If necessary, a second incision should be made on the left side of the fish from the initial point of the first incision toward the dorsal fin. The resulting flap should be folded back to observe the gonads. Ovaries appear whitish to greenish to golden brown and have a granular texture. Testes appear creamy white and have a smooth texture (Texas Water Commission, 1990). The sex of each fish should be recorded on the sample processing form.

7.2.2.5 Assessment of Morphological Abnormalities (Optional)—

Assessment of gross morphological abnormalities in finfish is optional. This assessment may be conducted in the field (see Section 6.3.1.5) or during initial inspection at the processing laboratory prior to filleting. States interested in documenting morphological abnormalities should consult Sinderman (1983) and review recommended protocols for fish pathology studies used in the Puget Sound Estuary Program (1990c) and those described by Goede and Barton (1990).

7.2.2.6 Scaling or Skinning—

To control contamination, separate sets of utensils and cutting boards should be used for skinning or scaling fish and for filleting fish. Fish with scales should be scaled and any adhering slime removed prior to filleting. Fish without scales (e.g., catfish) should be skinned prior to filleting. These fillet types are recommended because it is believed that they are most representative of the

edible portions of fish prepared and consumed by sport anglers. However, it is the responsibility of each program manager, in consultation with State fisheries experts, to select the fillet or sample type most appropriate for each target species based on the dietary customs of local populations of concern.

A fish is scaled by laying it flat on a clean glass or PTFE cutting board or on one that has been covered with heavy duty aluminum foil and removing the scales and adhering slime by scraping from the tail to the head using the blade edge of a clean stainless steel, ceramic, or titanium knife. Cross-contamination is controlled by rinsing the cutting board and knife with contaminant-free distilled water between fish. If an aluminum foil covered cutting board is used, the foil should be changed between fish. The skin should be removed from fish without scales by loosening the skin just behind the gills and pulling it off between knife blade and thumb or with pliers as shown in Figure 7-3.

Once the scales and slime have been scraped off or the skin removed, the outside of the fish should be washed with contaminant-free distilled water and it should be placed on a second clean cutting board for filleting.

7.2.2.7 Filleting—

Filleting should be conducted only by or under the supervision of an experienced fisheries biologist. If gloves are worn, they should be talc- or dust-free, and of non- contaminating materials. Prior to filleting, hands should be washed with Ivory soap and rinsed thoroughly in tap water, followed by distilled water (U.S. EPA, 1991d). Specimens should come into contact with noncontaminating surfaces only. Fish should be filleted on glass or PTFE cutting boards that are cleaned properly between fish or on cutting boards covered with heavy duty aluminum foil that is changed between fish (Puget Sound Estuary Program, 1990d, 1990e). Care must be taken to avoid contaminating fillet tissues with material released from inadvertent puncture of internal organs. **Note:** If the fillet tissue is contaminated by materials released from the inadvertent puncture of the internal organs during resection, the State may eliminate the fillet tissue as a sample or, alternatively, the fillet tissue should be rinsed in contaminant-free, deionized distilled water and blotted dry. Regardless of the procedure selected, a notation should be made in the sample processing record.

Ideally, fish should be filleted while ice crystals are still present in the muscle tissue. Therefore, if fish have been frozen, they should not be allowed to thaw completely prior to filleting. Fish should be thawed only to the point where it becomes possible to make an incision into the flesh (U.S. EPA, 1991d).

Clean, high-quality stainless steel, ceramic, or titanium utensils should be used to remove one or both fillets from each fish, as necessary. The general procedure recommended for filleting fish is illustrated in Figure 7-3 (U.S. EPA, 1991d).

Source: U.S. EPA, 1991d.

Figure 7-3. Illustration of basic fish filleting procedure.

The belly flap should be included in each fillet. Any dark muscle tissue in the vicinity of the lateral line should not be separated from the light muscle tissue that constitutes the rest of the muscle tissue mass. Bones still present in the tissue after filleting should be removed carefully (U.S. EPA, 1991d).

If both fillets are removed from a fish, they can be combined or kept separate for duplicate QC analysis, analysis of different analytes, or archival of one fillet. Fillets should be weighed (either individually or combined, depending on the analytical requirements) and the weight(s) recorded to the nearest gram on the sample processing record.

If fillets are to be homogenized immediately, they should be placed in a properly cleaned glass or PTFE homogenization container. If samples are to be analyzed for metals only, plastic homogenization containers may be used. To facilitate homogenization it may be necessary or desirable to chop each fillet into smaller pieces using a titanium or stainless steel knife prior to placement in the homogenization container.

If fillets are to be homogenized later, they should be wrapped in heavy duty aluminum foil and labeled with the sample identification number, the sample type (e.g., "F" for fillet), the weight (g), and the date of resection. If composite homogenates are to be prepared from only a single fillet from each fish, fillets should be wrapped separately and the designation "F1" and "F2" should be added to the sample identification number for each fillet. The individual fillets from each fish should be kept together. All fillets from a composite sample should be placed in a plastic bag labeled with the composite identification number, the individual sample identification numbers, and the date of resection and stored at ≤-20 °C until homogenization.

7.2.2.8 Preparation of Individual Homogenates—

To ensure even distribution of contaminants throughout tissue samples and to facilitate extraction and digestion of samples, the fillets from individual fish must be ground and homogenized prior to analysis. The fillets from an individual fish may be ground and homogenized separately, or combined, depending on the analytical requirements and the sample size.

Fish fillets should be ground and homogenized using an automatic grinder or high-speed blender or homogenizer. Large fillets may be cut into 2.5-cm cubes with high-quality stainless steel or titanium knives or with a food service band saw prior to homogenization. Parts of the blender or homogenizer used to grind the tissue (i.e., blades, probes) should be made of tantalum or titanium rather than stainless steel. Stainless steel blades and/or probes have been found to be a potential source of nickel and chromium contamination (due to abrasion at high speeds) and should be avoided.

Grinding and homogenization of tissue is easier when it is partially frozen (Stober, 1991). Chilling the grinder/blender briefly with a few chips of dry ice will also help keep the tissue from sticking to it (Smith, 1985).

The fillet sample should be ground until it appears to be homogeneous. The ground sample should then be divided into quarters, opposite quarters mixed together by hand, and the two halves mixed together. The grinding, quartering, and hand-mixing steps should be repeated at least two more times. If chunks of tissue are present at this point, the grinding and homogenization should be repeated. **Note:** Skin-on fillets are the fish fillet sample type recommended for use in State fish contaminant monitoring programs. However, skin-on fillets of some finfish species are especially difficult to homogenize completely. No chunks of tissue or skin should remain in the sample homogenate because these may not be extracted or digested efficiently and could bias the analytical results. If complete homogenization of skin-on fillets for a particular target species is a chronic problem or if local consumers are likely to prepare skinless fillets of the species, the State should consider analyzing skinless fillet samples. If the sample is to be analyzed for metals only, the ground tissue may be mixed by hand in a polyethylene bag (Stober, 1991). The preparation of each individual homogenate should be noted (marked with a check) on the sample processing record. At this time, individual homogenates may be either processed further to prepare composite homogenates or frozen separately and stored at ≤-20 °C (see Table 7-1).

7.2.2.9 Preparation of Composite Homogenates—

Composite homogenates should be prepared from equal weights of individual homogenates. The same type of individual homogenate (i.e., either single fillet or combined fillet) should always be used in a given composite sample.

If individual homogenates have been frozen, they should be thawed partially and rehomogenized prior to weighing and compositing. Any associated liquid should be kept as a part of the sample. The weight of each individual homogenate used in the composite homogenate should be recorded, to the nearest gram, on the sample processing record.

Each composite homogenate should be blended as described for individual homogenates in Section 7.2.2.8. The composite homogenate may be processed immediately for analysis or frozen and stored at ≤-20 °C (see Table 7-1).

The remainder of each individual homogenate should be archived at ≤-20 °C with the designation "Archive" and the expiration date recorded on the sample label. The location of the archived samples should be indicated on the sample processing record under "Notes."

It is essential that the weights of individual homogenates yield a composite homogenate of adequate size to perform all necessary analyses. Weights of individual homogenates required for a composite homogenate, based on the

number of fish per composite and the weight of composite homogenate recommended for analyses of all screening study target analytes (see Table 4-1), are given in Table 7-2. The total composite weight required for intensive studies may be less than that for screening studies if the number of target analytes is reduced significantly.

The recommended sample size of 200 g for screening studies is intended to provide sufficient sample material to (1) analyze for all recommended target analytes (see Table 4-1) at appropriate detection limits; (2) meet minimum QC requirements for the analyses of laboratory duplicate, matrix spike, and matrix spike duplicate samples (see Sections 8.3.3.4 and 8.3.3.5); and (3) allow for reanalysis if the QC control limits are not met or if the sample is lost. However, sample size requirements may vary among laboratories and the analytical methods used. Each program manager must consult with the analytical laboratory supervisor to determine the actual weights of composite homogenates required to analyze for all selected target analytes at appropriate detection limits.

Table 7-2. Weights (g) of Individual Homogenates Required for Screening Study Composite Homogenate Sample[a,b]

Number of fish per sample	Total composite weight		
	100 g (minimum)	200 g (recommended)	500 g (maximum)
3	33	67	167
4	25	50	125
5	20	40	100
6	17	33	84
7	14	29	72
8	13	25	63
9	11	22	56
10	10	20	50

[a]Based on total number of fish per composite and the total composite weight required for analysis in screening studies. The total composite weight required in intensive studies may be less if the number of target analytes is reduced significantly.

[b]Individual homogenates may be prepared from one or both fillets from a fish. A composite homogenate should be prepared only from individual homogenates of the same type (i.e., **either** from individual homogenates each prepared from a single fillet **or** from individual homogenates each prepared from both fillets).

7.2.3 Processing Turtle Samples

Processing in the laboratory to prepare individual turtle homogenate samples for analysis (diagrammed in Figure 7-4) involves

- Inspecting individual turtles
- Weighing individual turtles
- Removing edible tissues
- Determining the sex of each turtle (optional)
- Determining the age of each turtle (optional)
- Weighing edible tissue or tissues
- Homogenizing tissues
- Preparing individual homogenate samples
- Preparing aliquots of the individual homogenates for analysis
- Distributing frozen aliquots to one or more analytical laboratories.

Whole turtles should be shipped or brought to the sample processing laboratory from the field on wet or blue ice within 24 hours of sample collection. The recommended euthanizing method for turtles is freezing (Frye, 1994) and a minimum of 48 hours or more may be required for large specimens. Turtles that arrive on wet or blue ice or frozen (i.e., on dry ice) at the sample processing laboratory should be placed in a ≤-20 °C freezer for storage until resection can be performed. If rupture of internal organs is noted for an individual turtle, the specimen may be eliminated as a sample or, alternatively, the edible tissues should be rinsed in distilled deionized water and blotted dry.

Sample processing procedures are discussed in the following sections. Data from each procedure should be recorded directly in a bound laboratory notebook or on forms that can be secured in the laboratory notebook. An example sample processing record for individual turtle samples is shown in Figure 7-5.

7.2.3.1 Sample Inspection—

Turtles received for resection should be removed from the canvas or burlap collection bags and inspected carefully to ensure that they have not been compromised in any way (i.e., not properly preserved during shipment). Any specimen deemed unsuitable for further processing and analysis should be discarded and identified on the sample processing record.

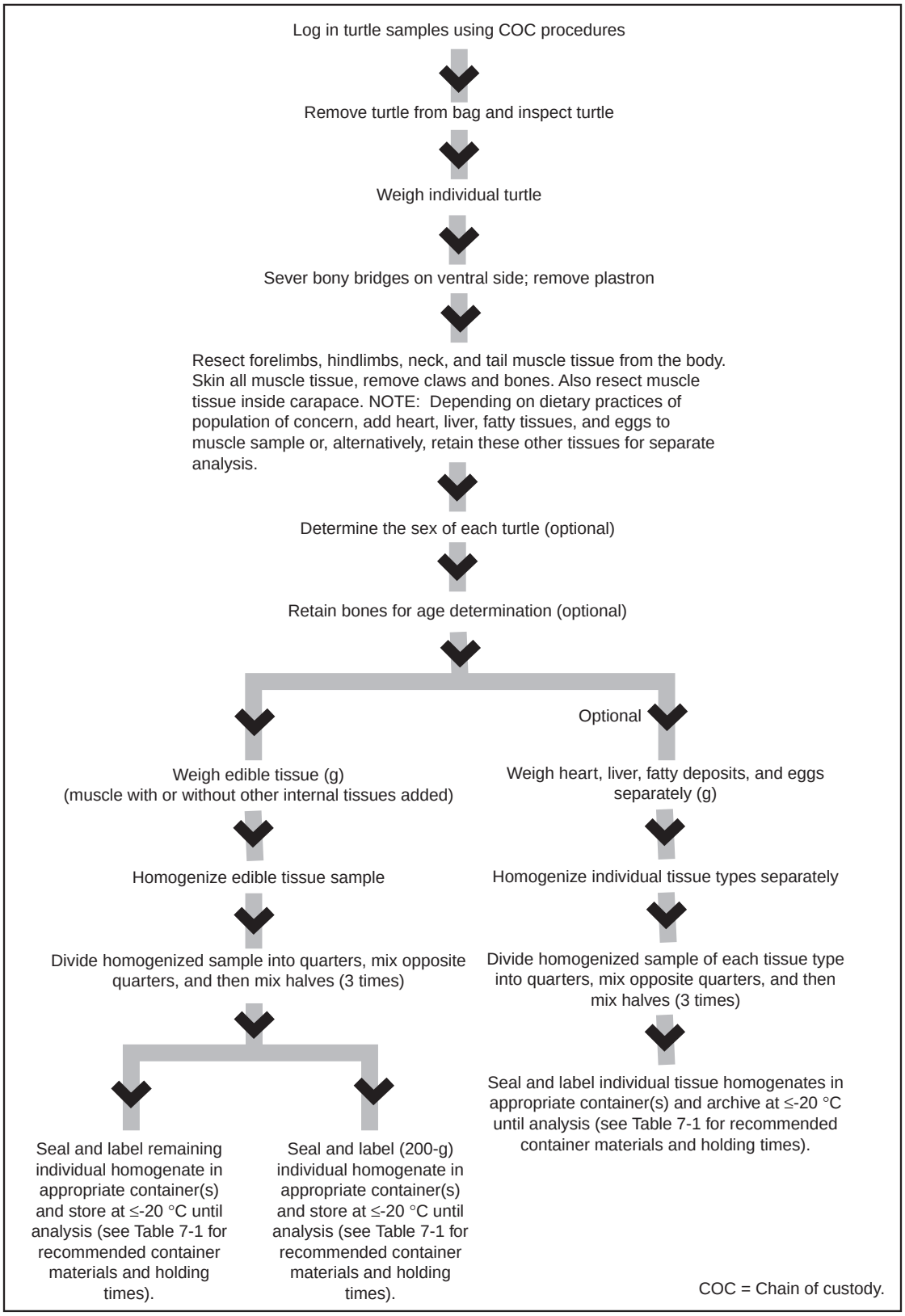


Figure 7-4. Preparation of individual turtle homogenate samples.

Figure 7-5. Example of a sample processing record for a contaminant monitoring program—individual turtle samples.

7.2.3.2 Sample Weighing—

A wet weight should be determined for each turtle. All samples should be weighed on balances that are properly calibrated and of adequate accuracy and precision to meet program data quality objectives. Balance calibration should be checked at the beginning and end of each weighing session and after every 20 weighings in a weighing session.

Turtles euthanized by freezing should be weighed in clean, tared, noncontaminating containers if they will thaw before the weighing can be completed. **Note:** Liquid from the thawed whole turtle sample will come not only from the muscle tissue but from the gut and body cavity, which may not be part of the desired edible tissue sample. Consequently, inclusion of this liquid with the sample may result in an overestimate of target analyte and lipid concentrations in the edible tissue homogenate. Nevertheless, it is recommended, as a conservative approach, that all liquid from the thawed whole turtle be kept in the container as part of the sample.

All weights should be recorded to the nearest gram on the sample processing record and/or in the laboratory notebook.

7.2.3.3 Removal of Edible Tissues—

Edible portions of a turtle should consist only of those tissues that the population of concern might reasonably be expected to eat. Edible tissues should be clearly defined in site-specific sample processing protocols. A brief description of the edible portions used should also be provided on the sample processing record. General procedures for removing edible tissues from a turtle are illustrated in Appendix I.

Resection should be conducted only by or under the supervision of an experienced fisheries biologist. If gloves are worn, they should be talc- or dust-free, and of noncontaminating materials. Prior to resection, hands should be washed with soap and rinsed thoroughly in tap water, followed by distilled water (U.S. EPA, 1991d). Specimens should come into contact with noncontaminating surfaces only. Turtles should be resected on glass or PTFE cutting boards that are cleaned properly between each turtle or on cutting boards covered with heavy duty aluminum foil that is changed between each turtle (Puget Sound Estuary Program, 1990d, 1990e). A turtle is resected by laying it flat on its back and removing the plastron by severing the two bony ridges between the fore and hindlimbs. Care must be taken to avoid contaminating edible tissues with material released from the inadvertent puncture of internal organs.

Ideally, turtles should be resected while ice crystals are still present in the muscle tissue. Thawing of frozen turtles should be kept to a minimum during tissue removal to avoid loss of liquids. A turtle should be thawed only to the point where it becomes possible to make an incision into the flesh (U.S. EPA, 1991d).

Clean, high-quality stainless steel, ceramic, or titanium utensils should be used to remove the muscle tissue and, depending on dietary or culinary practices of the population of concern, some of the other edible tissues from each turtle. The general procedure recommended for resecting turtles is illustrated in Figure 7-6.

Skin on the forelimbs, hindlimbs, neck, and tail should be removed. Claws should be removed from the fore and hindlimbs. Bones still present in the muscle tissue after resection should be removed carefully (U.S. EPA, 1991d) and may be used in age determination (see Section 7.2.3.5).

To control contamination, separate sets of utensils and cutting boards should be used for skinning muscle tissue and resecting other internal tissues from the turtle (e.g., heart, liver, fatty deposits, and eggs). These other tissue types are recommended for inclusion with the muscle tissue as part of the edible tissue sample because it is believed that they are most representative of the edible portions of turtles that are prepared and consumed by sport anglers and subsistence fishers. Alternatively, States may choose to analyze some of these other lipophilic tissues separately. It is the responsibility of each program manager, in consultation with State fisheries experts, to select the tissue sample type most appropriate for each target species based on the dietary customs of local populations of concern.

The edible turtle tissues should be weighed and the weight recorded to the nearest gram on the sample processing record. If the State elects to analyze the heart, liver, fatty deposits, or eggs separately from the muscle tissue, these other tissues should be weighed separately and the weights recorded to the nearest gram in the sample processing record.

If the tissues are to be homogenized immediately, they should be placed in a properly cleaned glass or PTFE homogenization container. If samples are to be analyzed for metals only, plastic homogenization containers may be used. To facilitate homogenization it may be necessary or desirable to chop each of the large pieces of muscle tissue into smaller pieces using a titanium or stainless steel knife prior to placement in the homogenization container.

If the tissues are to be homogenized later, they should be wrapped in heavy duty aluminum foil and labeled with the sample identification number, the sample type (e.g., "M" for muscle, "E" for eggs, or "FD" for fatty deposits), the weight (g), and the date of resection. The individual muscle tissue samples from each turtle should be packaged together and given an individual sample identification number. The date of resection should be recorded and the sample should be stored at ≤-20 °C until homogenization. **Note**: State staff may determine that the most appropriate sample type is muscle tissue only, with internal organ tissues analyzed separately (liver, heart, fatty deposits, or eggs). Alternatively, State staff may determine that the most appropriate sample type is muscle tissue with several other internal organs included as the turtle tissue sample. This latter sample type typically will provide a more conservative estimate of

Source: Hamerstrom, 1989.

Figure 7-6. Illustration of basic turtle resection procedure.

contaminant residues, particularly with respect to lipophilic target analytes (e.g., PCBs, dioxins, and organochlorine pesticides).

7.2.3.4 Sex Determination (Optional)—

Turtle sex should be determined during resection if it has not already been determined in the field. Once the plastron is removed, the ovaries or testes can be observed posterior and dorsal to the liver. Each ovary is a large egg-filled sac containing yellow spherical eggs in various stages of development (Ashley, 1962) (see Appendix I). Each testes is a spherical organ, yellowish in color, attached to the ventral side of each kidney. The sex of each turtle should be verified and recorded on the sample processing form.

7.2.3.5 Age Determination (Optional)—

Age provides a good indication of the duration of exposure to pollutants (Versar, 1982). Several methods have been developed for estimating the age of turtles (Castanet, 1994; Frazer et al., 1993; Gibbons, 1976). Two methods are appropriate for use in contaminant monitoring programs where small numbers of animals of a particular species are to be collected and where the animals must be sacrificed for tissue residue analysis. These methods include (1) the use of external annuli (scute growth marks) on the plastron and (2) the use of growth rings on the bones.

The surface of epidermal keratinous scutes on the plastron of turtle shells develops successive persistent grooves or growth lines during periods of slow or arrested growth (Zangerl, 1969). Because these growth rings are fairly obvious, they have been used extensively for estimating age in various turtle species (Cagle, 1946, 1948, 1950; Gibbons, 1968; Legler, 1960; Sexton, 1959). This technique is particularly useful for younger turtles where the major growth rings are more definitive and clear cut than in older individuals (Gibbons, 1976). However, a useful extension of the external annuli method is presented by Sexton (1959) showing that age estimates can be made for adults on which all annuli are not visible. This method may be performed by visually examining the plastron of the turtle during the resection, or the plastron may be tagged with the sample identification number of the turtle and retained for later analysis.

The use of bone rings is the second method that may be used to estimate age in turtles (Enlow and Brown, 1969; Peabody, 1961). Unlike the previous visual method, this method requires that the bones of the turtle be removed during resection and retained for later analysis. The growth rings appear at the surface or inside primary compacta of bone tissues. There are two primary methods for observing growth marks: either directly at the surface of the bone as in flat bones using transmitted or reflected light or inside the long bones using thin sections (Castanet, 1994; Dobie, 1971; Galbraith and Brooks, 1987; Hammer, 1969; Gibbons, 1976; Mattox, 1935; Peabody, 1961). The methods of preparation of whole bones and histological sections of fresh material for growth mark determinations are now routinely performed. Details of these methods can be

found in Castanet (1974 and 1987), Castanet et al. (1993), and Zug et al. (1986).

State staff interested in using either of these methods for age determination of turtles should read the review articles by Castanet (1994) and Gibbons (1976) for discussions of the advantages and disadvantages of each method, and the associated literature cited in these articles on turtle species of particular interest within their jurisdictions.

7.2.3.6 Preparation of Individual Homogenates—

To ensure even distribution of contaminants throughout tissue samples and to facilitate extraction and digestion of samples, the edible tissues from individual turtles must be ground and homogenized prior to analysis. The various tissues from an individual turtle may be ground and homogenized separately, or combined, depending on the sampling program's definition of edible tissues.

Turtle tissues should be ground and homogenized using an automatic grinder or high-speed blender or homogenizer. Large pieces of muscle or organ tissue (e.g., liver or fatty deposits) may be cut into 2.5-cm cubes with high-quality stainless steel or titanium knives or with a food service band saw prior to homogenization. Parts of the blender or homogenizer used to grind the tissue (i.e., blades, probes) should be made of tantalum or titanium rather than stainless steel. Stainless steel blades and/or probes have been found to be a potential source of nickel and chromium contamination (due to abrasion at high speeds) and should be avoided.

Grinding and homogenization of tissue is easier when it is partially frozen (Stober, 1991). Chilling the grinder/blender briefly with a few chips of dry ice will also help keep the tissue from sticking to it (Smith, 1985).

The tissue sample should be ground until it appears to be homogeneous. The ground sample should then be divided into quarters, opposite quarters mixed together by hand, and the two halves mixed together. The grinding, quartering, and hand-mixing steps should be repeated at least two more times. If chunks of tissue are present at this point, the grinding and homogenization should be repeated. No chunks of tissue should remain because these may not be extracted or digested efficiently and could bias the analytical results. This is particularly true when lipophilic tissues (e.g., fatty deposits, liver, or eggs) are not completely homogenized throughout the sample. Portions of the tissue sample that retain unhomogenized portions of tissues may exhibit higher or lower residues of target analytes than properly homogenized samples.

If the sample is to be analyzed for metals only, the ground tissue may be mixed by hand in a polyethylene bag (Stober, 1991). The preparation of each individual homogenate should be noted (marked with a check) on the sample processing record. At this time, individual homogenates may be frozen separately and stored at ≤-20 °C (see Table 7-1).

The remainder of each individual homogenate should be archived at ≤-20 °C with the designation "Archive" and the expiration date recorded on the sample label. The location of the archived samples should be indicated on the sample processing record under "Notes."

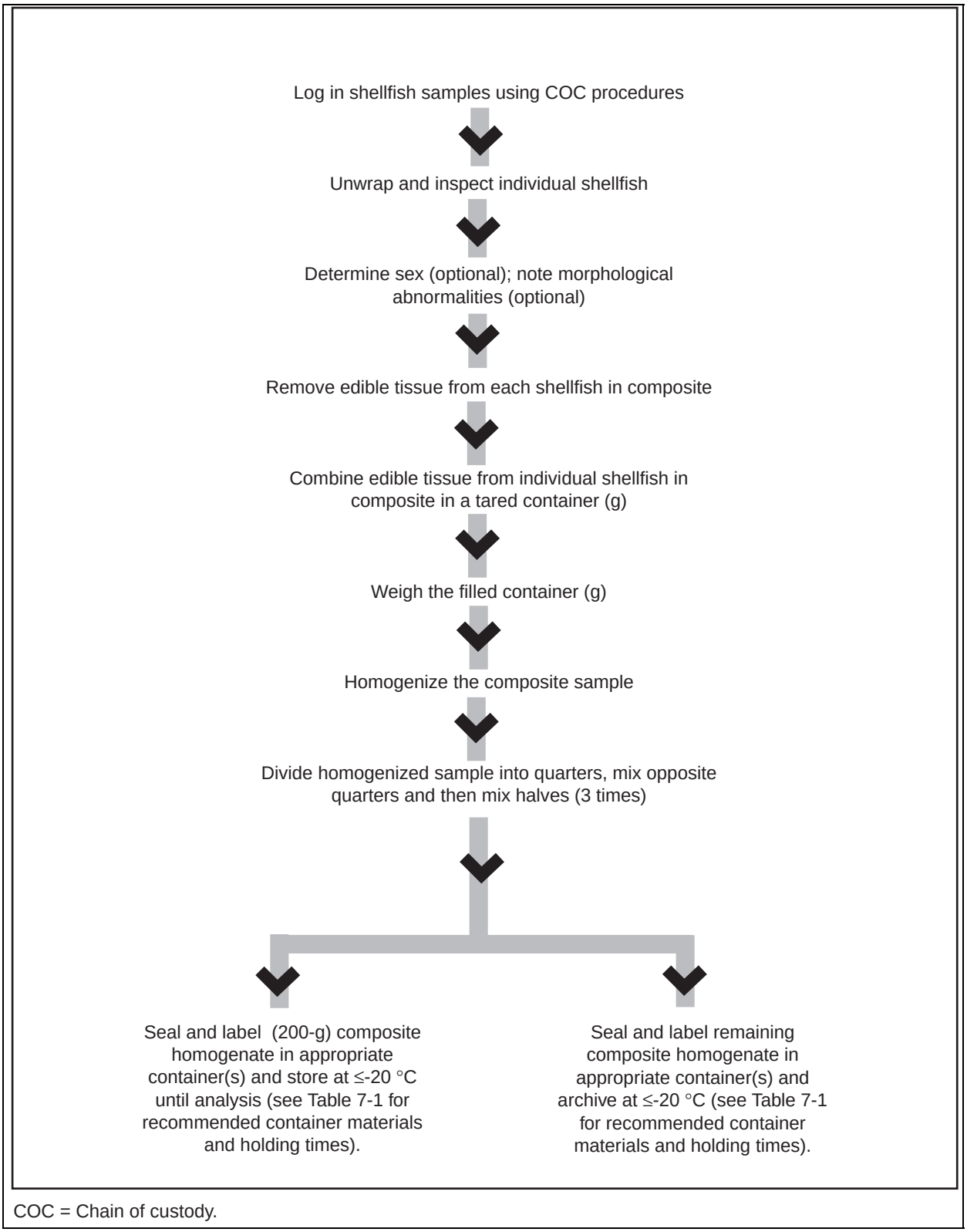
It is essential that the weight of individual homogenate samples is of adequate size to perform all necessary analyses. The recommended sample size of 200 g for screening studies is intended to provide sufficient sample material to (1) analyze for all recommended target analytes (see Table 4-1) at appropriate detection limits; (2) meet minimum QC requirements for the analyses of laboratory duplicate, matrix spike, and matrix spike duplicate samples (see Sections 8.3.3.4 and 8.3.3.5); and (3) allow for reanalysis if the QC control limits are not met or if the sample is lost. However, sample size requirements may vary among laboratories and the analytical methods used. Each program manager must consult with the analytical laboratory supervisor to determine the actual weights of homogenates required to analyze for all selected target analytes at appropriate detection limits. The total sample weight required for intensive studies may be less than that for screening studies if the number of target analytes is reduced significantly.

7.2.4 Processing Shellfish Samples

Laboratory processing of shellfish to prepare edible tissue composite homogenates for analysis (diagrammed in Figure 7-7) involves

- Inspecting individual shellfish
- Determining the sex of each shellfish (optional)
- Examining each shellfish for morphological abnormalities (optional)
- Removing the edible parts from each shellfish in the composite sample (3 to 50 individuals, depending upon the species)
- Combining the edible parts in an appropriate noncontaminating container
- Weighing the composite sample
- Homogenizing the composite sample
- Preparing aliquots of the composite homogenate for analysis
- Distributing frozen aliquots to one or more analytical laboratories.

Sample aliquotting and shipping are discussed in Section 7.3; all other processing steps are discussed in this section. Shellfish samples should be processed following the general guidelines in Section 7.2.1 to avoid contamination. In particular, it is recommended that separate composite



COC = Chain of custody.

Figure 7-7. Preparation of shellfish edible tissue composite homogenate samples.

homogenates be prepared for the analysis of metals and organics if resources allow. An example sample processing record for shellfish edible tissue composite samples is shown in Figure 7-8.

Shellfish samples should be shipped or brought to the sample processing laboratory either on wet or blue ice (if next-day delivery is assured) or on dry ice (see Section 6.3.3). Shellfish samples arriving on wet ice or blue ice should have edible tissue removed and should be frozen to ≤-20 °C within 48 hours after collection. Shellfish samples that arrive frozen (i.e., on dry ice) at the processing laboratory should be placed in a ≤-20 °C freezer for storage until edible tissue is removed.

7.2.4.1 Sample Inspection—

Individual shellfish should be unwrapped and inspected carefully to ensure that they have not been compromised in any way (i.e., not properly preserved during shipment). Any specimen deemed unsuitable for further processing and analysis should be discarded and identified on the sample processing record.

7.2.4.2 Sex Determination (Optional)—

The determination of sex in shellfish species is impractical if large numbers of individuals of the target species are required for each composite sample.

For bivalves, determination of sex is a time-consuming procedure that must be performed after shucking but prior to removal of the edible tissues. Once the bivalve is shucked, a small amount of gonadal material can be removed using a Pasteur pipette. The gonadal tissue must then be examined under a microscope to identify egg or sperm cells.

For crustaceans, sex also should be determined before removal of the edible tissues. For many species, sex determination can be accomplished by visual inspection. Sexual dimorphism is particularly striking in many species of decapods. In the blue crab, *Callinectes sapidus*, the female possesses a broad abdomen suited for retaining the maturing egg mass or sponge, while the abdomen of the male is greatly reduced in width. For shrimp, lobsters, and crayfish, sexual variations in the structure of one or more pair of pleopods are common.

States interested in determining the sex of shellfish should consult taxonomic keys for specific information on each target species.

7.2.4.3 Assessment of Morphological Abnormalities (Optional)—

Assessment of gross morphological abnormalities in shellfish is optional. This assessment may be conducted in the field (see Section 6.3.1.5) or during initial inspection at the processing laboratory prior to removal of the edible tissues. States interested in documenting morphological abnormalities should consult

Figure 7-8. Example of a sample processing record for shellfish contaminant monitoring program—edible tissue composites.

Sinderman and Rosenfield (1967), Rosen (1970), and Murchelano (1982) for detailed information on various pathological conditions in shellfish and review recommended protocols for pathology studies used in the Puget Sound Estuary Program (1990c).

7.2.4.4 Removal of Edible Tissue—

Edible portions of shellfish should consist only of those tissues that the population of concern might reasonably be expected to eat. Edible tissues should be clearly defined in site-specific sample processing protocols. A brief description of the edible portions used should also be provided on the sample processing record. General procedures for removing edible tissues from a variety of shellfish are illustrated in Appendix I.

Thawing of frozen shellfish samples should be kept to a minimum during tissue removal to avoid loss of liquids. Shellfish should be rinsed well with organics- and metal-free water prior to tissue removal to remove any loose external debris.

Bivalve molluscs (oysters, clams, mussels, and scallops) typically are prepared by severing the adductor muscle, prying open the shell, and removing the soft tissue. The soft tissue includes viscera, meat, and body fluids (Smith, 1985). Byssal threads from mussels should be removed with a knife before shucking and should not be included in the composite sample.

Edible tissue for **crabs** typically includes all leg and claw meat, back shell meat, and body cavity meat. Internal organs generally are removed. Inclusion of the hepatopancreas should be determined by the eating habits of the local population or subpopulations of concern. If the crab is soft-shelled, the entire crab should be used in the sample. Hard- and soft-shelled crabs must not be combined in the same composite (Smith, 1985).

Typically, **shrimp** and **crayfish** are prepared by removing the cephalothorax and then removing the tail meat from the shell. Only the tail meat with the section of intestine passing through the tail muscle is retained for analysis (Smith, 1985).

Edible tissue for **lobsters** typically includes the tail and claw meat. If the tomalley (hepatopancreas) and gonads or ovaries are consumed by local populations of concern, these parts should also be removed and analyzed separately (Duston et al., 1990).

7.2.4.5 Sample Weighing—

Edible tissue from all shellfish in a composite sample (3 to 50 individuals) should be placed in an appropriate preweighed and labeled noncontaminating container. The weight of the empty container (tare weight) should be recorded to the nearest gram on the sample processing record. All fluids accumulated during removal of edible tissue should be retained as part of the sample. As the edible portion of each shellfish is placed in the container, it should be noted on the

sample processing record. When the edible tissue has been removed from all shellfish in the composite, the container should be reweighed and the weight recorded to the nearest gram on the sample processing record. The total composite weight should be approximately 200 g for screening studies. If the number of target analytes is significantly reduced in intensive studies, a smaller composite homogenate sample may suffice (see Section 7.2.2.9). At this point, the composite sample may be processed for analysis or frozen and stored at ≤-20 °C (see Table 7-1).

7.2.4.6 Preparation of Composite Homogenates—

Composite samples of the edible portions of shellfish should be homogenized in a grinder, blender, or homogenizer that has been cooled briefly with dry ice (Smith, 1985). For metals analysis, tissue may be homogenized in 4-oz polyethylene jars (California Department of Fish and Game, 1990) using a Polytron equipped with a titanium generator. If the tissue is to be analyzed for organics only, or if chromium and nickel contamination are not of concern, a commercial food processor with stainless steel blades and glass container may be used. The composite should be homogenized to a paste-like consistency. Larger samples may be cut into 2.5-cm cubes with high-quality stainless steel or titanium knives before grinding. If samples were frozen after dissection, they can be cut without thawing with either a knife-and-mallet or a clean bandsaw. The ground samples should be divided into quarters, opposite quarters mixed together by hand, and the two halves mixed together. The quartering and mixing should be repeated at least two more times until a homogeneous sample is obtained. No chunks should remain in the sample because these may not be extracted or digested efficiently. At this point, the composite homogenates may be processed for analysis or frozen and stored at ≤-20 °C (see Table 7-1).

7.3 SAMPLE DISTRIBUTION

The sample processing laboratory should prepare aliquots of the composite homogenates for analysis, distribute the aliquots to the appropriate laboratory (or laboratories), and archive the remainder of each composite homogenate.

7.3.1 Preparing Sample Aliquots

Note: Because lipid material tends to migrate during freezing, frozen composite homogenates must be thawed and rehomogenized before aliquots are prepared (U.S. EPA, 1991d). Samples may be thawed overnight in an insulated cooler or refrigerator and then homogenized. Recommended aliquot weights and appropriate containers for different types of analyses are shown in Table 7-3. The actual sample size required will depend on the analytical method used and the laboratory performing the analysis. Therefore, the exact sample size required for each type of analysis should be determined in consultation with the analytical laboratory supervisor.

The exact quantity of tissue required for each digestion or extraction and analysis should be weighed and placed in an appropriate container that has been labeled with the aliquot identification number, sample weight (to the nearest 0.1 g), and the date aliquots were prepared (Stober, 1991). The analytical laboratory can then recover the entire sample, including any liquid from thawing, by rinsing the container directly into the digestion or extraction vessel with the appropriate solvent. It is also the responsibility of the processing laboratory to provide a sufficient number of aliquots for laboratory duplicates, matrix spikes, and matrix spike duplicates so that the QC requirements of the program can be met (see Sections 8.3.3.4 and 8.3.3.5), and to provide extra aliquots to allow for reanalysis if the sample is lost or if QC control limits are not met.

It is essential that accurate records be maintained when aliquots are prepared for analysis. Use of a carefully designed form is recommended to ensure that all the necessary information is recorded. An example of a sample aliquot record is shown in Figure 7-9. The composite sample identification number should be assigned to the composite sample at the time of collection (see Section 6.2.3.1) and carried through sample processing (plus "F1," "F2," or "C" if the composite homogenate is comprised of individual or combined fillets). The aliquot identification number should indicate the analyte class (e.g., MT for metals, OR for organics, DX for dioxins) and the sample type (e.g., R for routine sample; RS for a routine sample that is split for analysis by a second laboratory; MS1 and MS2 for sample pairs, one of which will be prepared as a matrix spike). For example, the aliquot identification number may be of the form WWWWW-XX-YY-ZZZ, where WWWWW is a 5-digit sample composite identification number; XX indicates individual (F1 or F2), or combined (C) fillets; YY is the analyte code; and ZZZ is the sample type.

Blind laboratory duplicates should be introduced by preparing two separate aliquots of the same composite homogenate and labeling one aliquot with a "dummy" composite sample identification. However, the analyst who prepares the laboratory duplicates must be careful to assign a "dummy" identification

Table 7-3. Recommended Sample Aliquot Weights and Containers for Various Analyses

Analysis	Aliquot weight (g)	Shipping/storage container
Metals	1-5	Polystyrene, borosilicate glass, or PTFE jar with PTFE-lined lid
Organics	20-50	Glass or PTFE jar with PTFE-lined lid
Dioxins/furans	20-50	Glass or PTFE jar with PTFE-lined lid

PTFE = Polytetrafluoroethylene (Teflon).

Figure 7-9. Example of a fish and shellfish monitoring program sample aliquot record.

number that has not been used for an actual sample and to indicate clearly on the processing records that the samples are blind laboratory duplicates. The analytical laboratory should not receive this information.

When the appropriate number of aliquots of a composite sample have been prepared for all analyses to be performed on that sample, the remainder of the composite sample should be labeled with "ARCHIVE" and the expiration date and placed in a secure location at ≤-20 °C in the sample processing laboratory. The location of the archived samples should be indicated on the sample aliquot record. Unless analyses are to be performed immediately by the sample processing laboratory, aliquots for sample analysis should be frozen at ≤-20 °C before they are transferred or shipped to the appropriate analytical laboratory.

7.3.2 Sample Transfer

The frozen aliquots should be transferred on dry ice to the analytical laboratory (or laboratories) accompanied by a sample transfer record such as the one shown in Figure 7-10. Further details on Federal regulations for shipping biological specimens in dry ice are given in Section 6.3.3.2. The sample transfer record may include a section that serves as the analytical laboratory COC record. The COC record must be signed each time the samples change hands for preparation and analysis.

Figure 7-10. Example of a fish and shellfish monitoring program sample transfer record.